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Request for grant of a patent

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1. Your reference	P101538GB
2. Patent application number *(The Patent Office will fill in this part)*	0228796.9 11 DEC 2002
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	Adjuvantix Limited Firth Court Western Bank SHEFFIELD S10 2TN GB
Patents ADP number *(if you know it)*	8521320001
If the applicant is a corporate body, give the country/state of its incorporation	GB
4. Title of the invention	Valency
5. Name of your agent *(if you have one)*	Harrison Goddard Foote
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	31 St Saviourgate YORK YO1 8NQ
Patents ADP number *(if you know it)*	~~14571001~~ 7914237002

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Claim *(s)*	6
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12. Name and daytime telephone number of person to contact in the United Kingdom   Dr Rob Docherty   01904 732120

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Valency

The invention relates to an adjuvant comprising a conjugate complex of antibody and antigen wherein said complex has low antibody valency and including methods to prepare said adjuvant.

The immune system is made up of lymphocytes which are able to recognise specific antigens. B lymphocytes recognise antigens in their native conformation through surface immunoglobulin receptors, and T lymphocytes recognise protein antigens that are presented as peptides along with self molecules known as major histocompatibility antigen (MHC), or human leukocyte antigen (HLA) in humans, on the surface of antigen presenting cells. Antigen presenting cells occur in different forms and may be distinguished into 'classical' antigen presenting cells, exemplified by macrophages and dendritic cells, and 'non-classical' antigen presenting cells, which includes B lymphocytes. T lymphocytes may be further subdivided into "cytotoxic T lymphocytes", which are able to kill virally infected target cells, and "T helper" lymphocytes. T helper lymphocytes have a regulatory function and are able to help B lymphocytes to produce specific antibody, or to help macrophages to kill intracellular pathogens.

Antibodies may exist in several forms, for example there are the main classes: IgM, IgG, IgA, IgD and IgE, each with differing 'effector' functions whereby the effect of the antibody is determined. Effector functions include complement fixation, (resulting in the stimulation of inflammatory responses) which can be activated upon the formation of immune complexes of antigen and antibody by IgM, IgA and IgG. Another example of an effector function is the triggering of mast cells by antigen, which is brought about by the cross-linking of surface IgE on mast cells, tethered there by occupancy of the high-affinity receptor for IgE FcR-epsilon-I (a receptor for the Fc region of IgE). For some of the antibody classes there are subclasses, (e.g. IgG in man is composed of four different subclasses known as IgG1, IgG2, IgG3 and

IgG4). The IgG subclasses differ markedly in abundance and in their effector functions.

One of the most important developments in the history of medicine is the advent of vaccines which are used to protect against a wide variety of infectious diseases. There are also vaccines in development for the treatment of various non-infectious diseases such as autoimmune and neurodegenerative diseases and various cancers. Many vaccines are produced by inactivated or attenuated pathogens which are injected into an individual. The immunised individual responds by producing both a humoral (antibody) and cellular (cytolytic T cells, CTL's) response. For example, some influenza vaccines are made by inactivating the virus by chemical treatment with formaldehyde, likewise the Salk polio vaccine comprises whole virus inactivated with propionolactone. For many pathogens (particularly bacteria), chemical or heat inactivation, while it may give rise to vaccine immunogens that confer protective immunity, also gives rise to side effects such as fever and injection site reactions. In the case of bacteria, inactivated organisms tend to be so toxic that side effects have limited the application of such crude vaccine immunogens (e.g. the cellular pertussis vaccine). Many modern vaccines are therefore made from protective antigens of the pathogen, separated by purification or molecular cloning from the materials that give rise to side-effects. These latter vaccines are known as 'subunit vaccines'.

The development of subunit vaccines (e.g. vaccines in which the immunogen is a purified protein) has been the focus of considerable research in recent years. The emergence of new pathogens (such as HIV and group-B streptococcus) and the growth of antibiotic resistance have created a need to develop new vaccines and to identify further candidate molecules useful in the development of subunit vaccines. Likewise the discovery of novel vaccine antigens from genomic and proteomic studies is enabling the development of new subunit vaccine candidates, particularly against bacterial pathogens and cancers. However, although subunit vaccines tend to avoid the side effects of killed or attenuated pathogen vaccines, their 'pure' status has separated from the 'danger signals' that are often associated with whole organism vaccines, and subunit vaccines do not always have adequate immunogenicity. Many

candidate subunit vaccines have failed in clinical trials in recent years, that might otherwise have succeeded were a suitable adjuvant available to enhance the immune response to the purified antigen.

We describe an adjuvant with improved efficacy. An adjuvant is a substance or procedure which augments specific immune responses to antigens by modulating the activity of immune cells. Examples of adjuvants include, by example only, Freunds adjuvant, muramyl dipeptides, liposomes. WO97/38711 and US02/0136722 discloses, amongst other things, CD28:antigen and CD40: antigen conjugates which act as adjuvants and result in enhanced immune responses directed to the antigen part of the conjugate.

CD28/CD40:antigen conjugates can be produced in a number of ways, and utilising a number of possible cross-linkers. Preferred methods of conjugation utilise so-called hetero-bifunctional cross-linkers, which have different functional groups at each end of the molecule, and thus their use can prevent direct antigen-antigen cross linking, or antibody-antibody cross-linking. However despite the advantages of these cross-linkers, in many cases conjugates can still be formed which contain more than one antibody molecule and more than one antigen molecule.

For example, if sulfo-SMCC and SATA are used as cross-linkers, one of the conjugate components is first maleimated using sulfoSMCC and the other has sulfhydryl groups attached using SATA. Both the maleimation and the sulfhydryl modification are on primary amines, of which there may be several on both the antibody and the antigen (amino-terminal residues (4 on each Ig molecule), and any lysine residues). It is possible therefore for any antigen to be attached to more than one antibody molecule, and for any antibody molecule to be attached to more than one antigen molecule. In this way large, covalently linked, complexes of antibody and antigen can be formed. The complexes formed during conjugation can be characterised using a number of different parameters:

i) overall size of the conjugate (molecular weight);

ii) ratio of antibody to antigen (weight:weight);

iii) ratio of antibody to antigen (mole: mole);

iv) mean number of antibody molecules in the conjugate; and

v) mean number of antigen molecules in the conjugate.

For any one antigen of known molecular weight, all of these parameters can be derived from (iv) and (v). However as antigens vary in size, the relationships between these values will vary, and thus the optimal forms of conjugates will vary between small (peptides), medium (proteins) and large (polysaccharide) antigens.

From *in vitro* experiments it is known that signalling through both CD40 and CD28 is enhanced by increasing the valency of the interaction. Thus, in order to achieve optimal B or T cell proliferation, anti-CD40 or anti-CD28 are adhered to the plastic of tissue culture plates prior to the addition of the cells, or are cross-linked through the use of anti-Fc antibodies adhered to the plastic, or even through the use of Fc receptor expressing cell lines, such as CD32 expressing L929 cells which are themselves adhered to the tissue culture plastic (Banchereau *et al* Science 1991 252 70-72.). Surprisingly, we find that, unlike proliferation induction *in vitro*, the adjuvant effects of the antibodies are not enhanced by increased multivalency. Indeed, the adjuvant effects are diminished when either of the antibodies are in a multivalent state.

According to an aspect of the invention there is provided an adjuvant comprising an isolated conjugate of a CD28 and/or CD40 antibody and at least one antigen wherein said conjugate consists of an oligomeric complex wherein the antibody valency of the complex does not exceed an average of about five antibody molecules per complex.

The formation of a "conjugate" is by any means which results in a conjugation crosslinking or association of antigen with antibody. In a further alternative embodiment of the invention the conjugation or association of antigen with the adjuvant antibody may be achieved by particulate formulation e.g. using micro or

nanoparticulate materials made of biocompatible polymers such as poly lactide-glycolide, alum or liposomal formulations.

In a preferred embodiment of the invention said complex consists an average of one to four antibody molecules per complex.

In a further preferred embodiment of the invention said complex consists an average of one to three antibody molecules per complex. Preferably one to two antibody molecules per complex.

According to a further aspect of the invention there is provided a vaccine composition comprising a conjugate according to the invention.

In a preferred embodiment of the invention said composition further comprises a carrier.

In a further preferred embodiment of the invention said composition further comprises a second adjuvant.

In a yet further preferred embodiment of the invention said composition comprises a mixture of a CD40 conjugate and a CD28 conjugate as herein described.

The term carrier are construed in the following manner. A carrier is an immunogenic molecule which, when bound to a second molecule augments immune responses to the latter. Some antigens are not intrinsically immunogenic (i.e. not immunogenic in their own right) yet may be capable of generating antibody responses when associated with a foreign protein molecule such as keyhole-limpet haemocyanin or tetanus toxoid. Such antigens contain B-cell epitopes but no T cell epitopes. The protein moiety of such a conjugate (the "carrier" protein) provides T-cell epitopes which stimulate helper T-cells that in turn stimulate antigen-specific B-cells to differentiate into plasma cells and produce antibody against the antigen. Helper T-cells can also

stimulate other immune cells such as cytotoxic T-cells, and a carrier can fulfil an analogous role in generating cell-mediated immunity as well as antibodies. Certain antigens which lack T-cell epitopes, such as polymers with a repeating B-cell epitope (e.g. bacterial polysaccharides), are intrinsically immunogenic to a limited extent. These are known as T-independent antigens. Such antigens benefit from association with a carrier such as tetanus toxoid, under which circumstance they elicit much stronger antibody responses. Carrier conjugation of bacterial polysaccarides is used to produce a number of 'conjugate vaccines' against bacterial infections such as *Haemophilus influenzae* (Hib) and group-C meningococci.

In a preferred embodiment of the invention said antigen is a T-cell dependent antigen.

In an alternative preferred embodiment of the invention said antigen is a T- cell independent antigen.

In a preferred embodiment of the invention said antigen is derived from a pathogenic bacterium.

Preferably said antigen is derived from a bacterial species selected from the group consisting of: *Staphylococcus aureus; Staphylococcus epidermidis; Enterococcus faecalis; Mycobacterium tuberculsis; Streptococcus group B; Streptoccocus pneumoniae; Helicobacter pylori; Neisseria gonorrhea; Streptococcus group A; Borrelia burgdorferi; Coccidiodes immitis; Histoplasma sapsulatum; Neisseria meningitidis ; Shigella flexneri; Escherichia coli; Haemophilus influenzae.*

In an alternative preferred embodiment of the invention said antigen is derived from a viral pathogen.

Preferably said antigen is derived from a viral pathogen selected from the group consisting of: Human Immunodeficiency Virus (HIV1 & 2); Human T Cell Leukaemia Virus (HTLV 1 & 2); Ebola virus; human papilloma virus (e.g. HPV-2, HPV-5, HPV-8 HPV-16, HPV-18, HPV-31, HPV-33, HPV-52, HPV-54 and HPV-56); papovavirus; rhinovirus; poliovirus; herpesvirus; adenovirus; Epstein Barr virus; influenza virus, hepatitis B and C viruses.

In a further preferred embodiment of the invention said antigen is derived from a parasitic pathogen.

In a yet further preferred embodiment of the invention said antigen is derived from a parasitic pathogen is *Trypanosoma spp, Schistosoma spp* or *Plasmodium spp.*

In a further preferred embodiment of the invention said antigen is derived from a fungal pathogen.

In a preferred embodiment of the invention said antigen is derived from a fungal pathogen which is of the genus *Candida spp*, preferably the species *Candida albicans*.

In a further preferred embodiment of the invention said antigen is a tumour specific antigen.

In a yet preferred embodiment of the invention said antigen is an addictive drug.

In further preferred embodiment of the invention said drug is selected from the group consisting of: cocaine; nicotine; or heroin.

According to a further aspect of the invention there is provided a method to immunise an animal to an antigen, comprising administering an effective amount of a

conjugate according to the invention sufficient to stimulate an immune response to said antigen.

In a preferred method of the invention said animal is human.

In an alternative preferred method of the invention said animal is selected from the group consisting of: mouse; rat; hamster; goat; cow, horse, pig, dog, cat or sheep.

In a further preferred method of the invention said immune response is the production of antibodies to said conjugate.

In an alternative preferred method of the invention said immune response is the production of T-helper cells which recognise the antigen part of said conjugate.

A preferred route of administration is intradermal, intramuscular or intranasal, however the immunisation method is not restricted to a particular mode of administration.

According to a yet further aspect of the invention there is provided an antibody obtainable by the method according to the invention.

In a preferred embodiment of the invention said antibody is a therapeutic antibody.

In a further preferred embodiment of the invention said antibody is a diagnostic antibody. Preferably said diagnostic antibody is provided with a label or tag.

In a preferred embodiment of the invention said antibody is a monoclonal antibody or binding fragment thereof. Preferably said antibody is a humanised or chimeric antibody.

A chimeric antibody is produced by recombinant methods to contain the variable region of an antibody with an invariant or constant region of a human antibody.

A humanised antibody is produced by recombinant methods to combine the complementarity determining regions (CDRs) of an antibody with both the constant (C) regions and the framework regions from the variable (V) regions of a human antibody.

Chimeric antibodies are recombinant antibodies in which all of the V-regions of a mouse or rat antibody are combined with human antibody C-regions. Humanised antibodies are recombinant hybrid antibodies which fuse the complimentarity determining regions from a rodent antibody V-region with the framework regions from the human antibody V-regions. The C-regions from the human antibody are also used. The complimentarity determining regions (CDRs) are the regions within the N-terminal domain of both the heavy and light chain of the antibody to where the majority of the variation of the V-region is restricted. These regions form loops at the surface of the antibody molecule. These loops provide the binding surface between the antibody and antigen.

Antibodies from non-human animals provoke an immune response to the foreign antibody and its removal from the circulation. Both chimeric and humanised antibodies have reduced antigenicity when injected to a human subject because there is a reduced amount of rodent (i.e. foreign) antibody within the recombinant hybrid antibody, while the human antibody regions do not ellicit an immune response. This results in a weaker immune response and a decrease in the clearance of the antibody. This is clearly desirable when using therapeutic antibodies in the treatment of human diseases. Humanised antibodies are designed to have less "foreign" antibody regions and are therefore thought to be less immunogenic than chimeric antibodies.

In a further preferred embodiment of the invention said antibodies are opsonic antibodies.

Phagocytosis is mediated by macrophages and polymorphic leukocytes and involves the ingestion and digestion of micro-organisms, damaged or dead cells, cell debris, insoluble particles and activated clotting factors. Opsonins are agents which facilitate the phagocytosis of the above foreign bodies. Opsonic antibodies are therefore antibodies which provide the same function. Examples of opsonins are the Fc portion of an antibody or compliment C3. Antibodies raised by immunisation and in the form of an immune complex with antigen may bring about opsonisation via the fixation of complement on the antigen, or molecules in its immediate microenvironment.

In a further aspect of the invention there is provided a method for preparing a hybridoma cell-line producing monoclonal antibodies according to the invention comprising the steps of:

i) immunising an immunocompetent mammal with a conjugate, composition, nucleic acid or vector according to the invention;
ii) fusing lymphocytes of the immunised immunocompetent mammal with myeloma cells to form hybridoma cells;
iii) screening monoclonal antibodies produced by the hybridoma cells of step (ii) for binding activity to the antigen of the conjugate according to the invention;
iv) culturing the hybridoma cells to proliferate and/or to secrete said monoclonal antibody; and
v) recovering the monoclonal antibody from the culture supernatant.

Preferably, said immunocompetent mammal is a rodent, for example a mouse, rat or hamster.

According to a further aspect of the invention there is provided a hybridoma cell-line obtainable by the method according to the invention.

According to a further aspect of the invention there is provided a method to crosslink an antibody, wherein said antibody is capable of binding a CD28 or CD40 receptor polypeptide and at least one antigen characterised in that reaction conditions are provided which select for conjugates with low antibody valency.

According to a yet further aspect of the invention there is provided a method to prepare a conjugate according to the invention comprising fractionation of a conjugation reaction mixture.

In a preferred method of the invention said fractionation comprises the following steps:

i) providing a reaction mixture consisting of a heterogeneous crosslinked antibody: antigen conjugate complex;
ii) separating the reaction mixture into fractions containing conjugates of defined size; and optionally
iii) isolating conjugates with a desired antibody valency.

In a preferred method of the invention said fraction contains a conjugate complex with an antibody valency of about on average five antibody molecules per complex.

In a further preferred method of the invention said fraction contains a complex with an antibody valency of between one to four antibodies per complex.

In a yet further preferred embodiment of the invention said fraction contains a complex with an antibody valency of between one and three antibodies per complex.

In a further preferred embodiment of the invention said fraction contains a complex of two antibody molecules, preferably said conjugate is a single antibody linked to at least one antigen.

In a preferred method of the invention said method is selected from the group consisting of: a size exclusion chromatographic method; an affinty chromatographic method; a differential precipitation method.

According to a further aspect of the invention there is provided a conjugate obtainable by the fractionation method according to the invention.

An embodiment of the invention will now be provided by example only and with reference to the following methods.

Materials and Methods

The antibody valency of the conjugates can be varied in a very large number of ways, indeed there are a large number of possible ways to perform the conjugations which will be known to those skilled in the art. The following are included by way of example only.

Alteration of the degree of derivatisation of the antigen.

One means of cross-linking antigens with antibodies is to maleimate the antigen, for instance using sulfo-SMCC, and subsequently to react the maleimated antigen with a thiolated antibody (antibody can be thiolated using SATA or SPDP.

The degree of maleimation of the antigen can be altered by changing the relative concentrations of sulfo-SMCC (sulfo-succinimIdyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate) and antigen in the reaction.

Another method of producing conjugates allows an accurate measurement of the degree of derivatisation of the antigen to be determined. The cross-linker SPDP (succinimidyl 3-(2-pyridyldithio)-propionate) can be used to thiolate the antigen. SPDP reacts at pH 7-9 with an amine containing antigen, yielding a mixed disulfide.

Subsequently, upon reduction with dithiothreitol, a 2-pyridinethione chromophore is released and a sulfhydryl group remains on the protein. From the amount of chromophore released (as determined by absorbance) it is possible to calculate the mean ratio of derivatisation on the antigen. Of course if this ratio were, for example, 3 sulfhydryl groups per antigen molecule, the subsequent conjugates with sulfo-SMCC maleimated antibody could not possibly have an antibody valency greater than 3. Thus control of the degree of derivatisation and therefore the mean number of thiol residues per antigen molecule could limit the antibody valency of the conjugates.

Another means of controlling valency would be to alter the number of reactive groups present in the antigen. Thus, for instance SPDP or SATA (among other cross-linkers) might be used to add sulfhydryl groups to the antigen. These cross-linkers both react with primary amines, therefore reaction can be with the amino-terminal residue, or with lysine residues elsewhere in the protein. It is possible to remove some lysine residues from a recombinant antigen by site-directed mutagenesis. It would of course be possible to remove lysine residues from a peptide by altering the synthesis. Of course if the antigenic sequences were being altered it would be important to ascertain that important epitopes were not being removed by this procedure.

Alteration of Antigen:antibody ratios in the reaction of the derivatised proteins.

The relative ratios of derivatised antigen, and antibody in the reaction mix can be altered, and will have effects on the kinds of conjugate formed which will also be dependent upon the degree of derivatisation and the relative sizes of the two components.

Purification of conjugates of different sizes

The conjugates produced can be purified by size fractionation. For example conjugates of glycoprotein D and antibody of between 200kDa and 400kDa could be separated from larger conjugates by gel filtration. Such conjugates could contain no more than two antibody molecules per conjugate (of approximately 150kDa each).

An alternative method to select conjugates of low valency rather than small size, would be to deplete high valency conjugates by affinity chromatography on SepharoseCL4B bearing Fc-gamma-receptor-IIb extracellular domain. Only high valency conjugates will stick to the column, or alternatively under isocratic conditions of 0.15M NaCl pH 7.4 10mM Na PO4 buffer, the species will elute in order of valency, i.e. low valency first (unadsorbed or weakly absorbed occurring in the 'void volume' of the column or soon thereafter.

Sizes of the purified conjugates can be assessed by gel filtration against known standards, or in some circumstances by polyacrylamide gel electrophoresis. Activity of the conjugates must then also be determined, most importantly regarding retention of antibody binding to either CD40 or CD28, and retention of antigencity of the antigen indicating that epitopes are intact. One method to verify these low activities would be to use flow cytometric staining of CD40 or CD28 expressing cells. Conjugates are added to the cells in PBS and incubated for 30min on ice. Cells are then washed and an antibody (either monoclonal or polyclonal) against the antigen added for 30min on ice. The antibody is then detected using a fluorescently labelled second antibody. Only conjugates with antibody binding (to CD40 or CD28) and antigenic epitopes still intact will give positive staining, and will be ready for assessment of immunogenicity.

<u>Phage PEG precipitation/purification</u>

It is known that PEG at 15% will precipitate free IgG from serum. Therefore a lower concentration would be appropriate for complexes such as is used to precipitate immune complexes, or larger molecules such as phage virions below.

1. add 30 ml of phage stock to SS-34 Oakridge tube.

2. add 7.5 ml 20 % PEG-8000/2.5 M NaCl.

3. incubate on ice for 30 minutes or longer.

4. spin down phage @ 11K for 20 minutes.

5. respin 2-3x to remove all of PEG solution (using a micro-pipet tip facilitates removal of all solution).

6. resuspend phage in STE (500-1000 ul).

7. transfer to eppendorf and spin @ 14K for 10 minutes.

8. transfer supernatant to new eppendorf and label.

9. titer phage.

STE: for 100 ml add 1 ml 1 M Tris (pH 8), 0.2 ml 0.5 M EDTA (pH 8), 2 ml 5 M NaCl. Autoclave.

PEG: for 100 ml add 20 gm PEG-8000 and 14.6 gm NaCl, filter sterilize.

Claims

1. An adjuvant comprising an isolated conjugate of a CD28 and/or CD40 antibody and at least one antigen wherein said conjugate consists of an oligomeric complex wherein the antibody valency of the complex does not exceed an average of about five antibody molecules per complex.

2. An adjuvant comprising an isolated conjugate of a CD28 and/or CD40 antibody and at least one antigen wherein said conjugate consists an average of one to four antibody molecules per complex.

3. An adjuvant according to Claim 2 wherein said complex consists an average of one to three antibody molecules per complex.

4. An adjuvant according to Claim 2 or 3 wherein said complex consists of one to two antibody molecules per complex.

5. A vaccine composition comprising a conjugate according to any of Claims 1-4.

6. A vaccine according to Claim 5 wherein said composition further comprises a carrier.

7. A vaccine according to Claim 5 or 6 wherein said composition further comprises a second adjuvant.

8. An vaccine according to Claim 7 wherein said composition comprises a mixture of a CD40 adjuvant and a CD28 adjuvant according to any of Claims 1-4.

9. An adjuvant according to any of Claims 1-4 wherein said antigen is a T-cell dependent antigen.

10. An adjuvant according to any of Claims 1-4 wherein said antigen is a T- cell independent antigen.

11. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from a pathogenic bacterium.

12. An adjuvant according to Claim 11 wherein said antigen is derived from a bacterial species selected from the group consisting of: *Staphylococcus aureus; Staphylococcus epidermidis; Enterococcus faecalis; Mycobacterium tuberculsis; Streptococcus group B; Streptoccocus pneumoniae; Helicobacter pylori; Neisseria gonorrhea; Streptococcus group A; Borrelia burgdorferi; Coccidiodes immitis; Histoplasma sapsulatum; Neisseria meningitidis ; Shigella flexneri; Escherichia coli; Haemophilus influenzae.*

13. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from a viral pathogen.

14. An adjuvant according to Claim 13 wherein said antigen is derived from a viral pathogen selected from the group consisting of: Human Immunodeficiency Virus (HIV1 & 2); Human T Cell Leukaemia Virus (HTLV 1 & 2); Ebola virus; human papilloma virus (e.g. HPV-2, HPV-5, HPV-8 HPV-16, HPV-18, HPV-31, HPV-33, HPV-52, HPV-54 and HPV-56); papovavirus; rhinovirus; poliovirus; herpesvirus; adenovirus; Epstein Barr virus; influenza virus, hepatitis B and C viruses.

15. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from a parasitic pathogen.

16. An adjuvant according to Claim 15 wherein said antigen is derived a parasistic pathogen selected from the group consisting of: *Trypanosoma spp, Schistosoma spp* or *Plasmodium spp*.

17. An adjuvant according to Claim 9 or 10 wherein said antigen is derived from a fungal pathogen.

18. An adjuvant according to Claim 17 wherein said antigen is derived from a fungal pathogen which is of the genus *Candida spp*.

19. An adjuvant according to Claim 9 or 10 wherein said antigen is a tumour specific antigen.

20. An adjuvant according to Claim 19 wherein said antigen is a ganglioside antigen.

21. An adjuvant according to Claim 19 or 20 wherein said antigen is MUC-1.

22. An adjuvant according to Claim 9 or 10 wherein said antigen is a hormone or a hormone receptor.

23. An adjuvant according to Claim 22 wherein said antigen is the N-methyl-D aspartate receptor, or part thereof.

24. An adjuvant according to Claim 9 or 10 wherein said antigen is a prion protein.

25. An adjuvant according to Claim 25 wherein said antigen is an amyloid protein.

26. An adjuvant according to Claim 25 wherein said antigen is amyloid β or part thereof.

27. An adjuvant according to Claim 9 or 10 wherein said antigen is a sperm antigen.

28. An adjuvant according to Claim 9 or 10 wherein said antigen is an addictive drug.

29. An adjuvant according to Claim 28 wherein said drug is selected from the group consisting of: cocaine; nicotine; or heroin.

30. A method to immunise an animal to an antigen, comprising administering an effective amount of adjuvant or vaccine according to any of Claims 1-29 sufficient to stimulate an immune response to said antigen.

31. A method according to Claim 30 wherein said animal is human.

32. A method according to Claim 30 wherein said animal is selected from the group consisting of: mouse; rat; hamster; goat; cow, horse, pig, dog, cat or sheep.

33. A method according to any of Claims 30-32 wherein said route of administration is intradermal, intramuscular or intranasal.

34. A method according to Claim 33 wherein said route is intranasal.

35. An antibody obtainable by the method according to any of Claims 30-34.

36. An antibody according to Claim 35 wherein said antibody is a monoclonal antibody or binding fragment thereof.

37 An antibody according to Claim 36 wherein said antibody is a humanised antibody.

38. An antibody according to Claim 36 wherein said antibody is a chimeric antibody.

39. An antibody according to Claim 36-38 wherein said antibody is an opsonic antibody.

40. A method for preparing a hybridoma cell-line producing monoclonal antibodies according to Claim 36 comprising the steps of:

i) immunising an immunocompetent mammal with a conjugate or, composition according 1-29;

ii) fusing lymphocytes of the immunised immunocompetent mammal with myeloma cells to form hybridoma cells;

iii) screening monoclonal antibodies produced by the hybridoma cells of step (ii) for binding activity to the antigen of the conjugate;

iv) culturing the hybridoma cells to proliferate and/or to secrete said monoclonal antibody; and

v) recovering the monoclonal antibody from the culture supernatant.

41. A hybridoma cell-line obtainable by the method according to Claim 40.

42. A method to crosslink an antibody, wherein said antibody is capable of binding a CD28 or CD40 receptor polypeptide, and at least one antigen characterised in that reaction conditions are provided which select for conjugates with low antibody valency.

43. A method to prepare an adjuvant according to any of Claims 1-4 or 9-29 comprising fractionation of a conjugation reaction mixture.

44. A method according to Claim 43 said fractionation comprises the following steps:

i) providing a reaction mixture consisting of a heterogeneous crosslinked antibody: antigen conjugate complex;

ii) separating the reaction mixture into fractions containing conjugates of defined size; and optionally

iii) isolating conjugates with a desired antibody valency.

45. A method according to Claim 44 wherein said fraction contains a conjugate complex with an antibody valency of about on average five antibody molecules per complex.

46. A method according to Claim 44 wherein said fraction contains a complex with an antibody valency of between one to four antibodies per complex.

47. A method according to Claim 44 whereinsaid fraction contains a complex with an antibody valency of between one and three antibodies per complex.

48. A method according to Claim 44 wherein said fraction contains a complex of two antibody molecules.

49. A method according to Claim 44 wherein said conjugate is a single antibody linked to at least one antigen.

50. A conjugate obtainable by the fractionation method according to any of Claims 43-49.

ABSTRACT

Valency

We describe antibody: antigen conjugates which consist of a complex which complex contains low antibody binding valency and including methods to prepare and isolate said complexes.